ICAFectin®-mRNA : Reagent for RNA transfection in primary and stem cells

Product description

- New synthetic derivative of natural compound.
- Particularly suitable for primary and stem cell transfection.
- Outstanding transfection efficiency for a wide variety of cell lines.
- Absence of toxicity at the effective concentrations.
- The ICAFectin®-mRNA/RNA complex must be prepared in medium that does not contain serum (Opti MEM is recommended) even if cells are transfected in the presence of serum.
- Removal of transfection complex is not needed.
- Adherent cells are equally transfected either with forward or reverse transfection procedures.
- Suspension cells are transfected following the specific procedure described herein.
- No need to keep complexes on ice during transfection.
- Storage at +2 to +8°C.
- Easy handling.
- Excellent reproducibility.
- Using standard experimental conditions, 500 µL of ICAFectin®-mRNA transfects over 330 wells in a 24-well format.
- For research purposes only. Not intended for animal or human therapeutic or diagnostic use.

Important note for transfection : Do not include serum and antibiotics during the formation of the ICAFectin®-mRNA/RNA complexes. For optimal transfection efficiency, we recommend Opti MEM I for the ICAFectin®-mRNA/RNA complex formation.

Transfection of adherent cells

Forward Transfection Procedure

Use the following procedure to transfect adherent cells in a 24-well format. For other formats, see **table 2** Scaling up/down Transfections.

Cell Preparation

One day before transfection, plate cells in 1 mL complete growth medium so that cells reach 70-80% confluence at the time of transfection (0.5 – 2 x 10^5 cells per well).

ICAFectin®-mRNA / RNA complex preparation

All amounts and volumes are given to transfect one well in a 24-well format.

1. Thirty minutes before transfection, remove growth medium and add 500 µL fresh medium with or without serum (depending on the cells).
2. Dilute 0.25 µg of RNA (in a maximal volume of 5 µL H2O) in 50 µL of Opti-MEM I without serum. Mix gently.

*Note : **Do not use** the serum-free culture medium in which cells were grown.*

3. Vortex ICAFectin®-mRNA before use. Dilute 1.5 µL of ICAFectin®-mRNA in 50 µL of Opti-MEM without serum. Mix gently.

*Note : **Do not use** the serum-free culture medium in which cells were grown.*

4. Combine the diluted ICAFectin®-mRNA (51.5 µL) with the diluted RNA (55 µL) by pipetting up and down five times and briefly vortexing.
5. Mix gently and incubate 15 minutes at room temperature.
6. Add the entire volume of complexes (106.5 µL) drop-wise to each well containing cells and 500 µL of fresh medium with or without serum (depending on the cells). Swirl the plate to ensure the homogeneous distribution of complexes.
7. Incubate cells with transfection complexes under their normal condition growth until analysis. Medium may be supplemented with 50 µL serum 100% or changed (depending on the cells) after 2 to 4 hours if cells have been incubated without serum.

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Reverse Transfection procedure

Use the following procedure to transfect RNA the day of seeding cells in wells. Reverse transfection gains one day. Amounts and volume are given to transfect one well in a 24-well format.

ICAFectin®-mRNA / RNA complex preparation

1. Dilute 0.25 µg of RNA (in a maximal volume of 5 µL H2O) in 50 µL of Opti-MEM without serum. Mix gently.

*Note : **Do not use** the serum-free culture medium in which cells were grown*

2. Vortex ICAFectin®-mRNA before use. Dilute 1.5 µL of ICAFectin®-mRNA in 50 µL of Opti-MEM without serum. Mix gently.

*Note : **Do not use** the serum-free culture medium in which cells were grown.*

3. Combine the diluted ICAFectin®-mRNA (51.5 µL) with the diluted RNA (55 µL) by pipetting up and down five times and briefly vortexing.
4. Mix gently and incubate 15 minutes at room temperature.
5. Add the entire volume of complexes (106.5 µL) drop-wise to each well and then seed 1-4 x 10^5 cells per well in 500 µL of fresh medium with or without serum (depending on the cells). Swirl the plate to ensure the homogeneous distribution of cells.
6. Incubate cells with transfection complexes under their normal growth conditions until analysis. Medium may be supplemented with 50 µL serum 100% or changed (depending on the cells) after 2 to 4 hours if cells have been incubated without serum.

Optimizing transfection

To obtain the highest transfection efficiency, transfect in medium that does not contain serum and optimize transfection by varying RNA quantity, ICAFectin®-mRNA amount and cell density.

For initial optimization in a 24-well format, use these six ICAFectin®-mRNA/RNA ratios (µL/µg).

Prepare complexes for a single well of a 24-well format as described in **table 1**.

Add the entire volume of complexes to each well.

Ratio (µL/µg)	RNA amount (µg) in 5µL H_2O	ICAFectin®-mRNA volume (µL)
1/0.25	0.25	1
1.5/0.25	**0.25**	**1.5**
2.5/0.25	0.25	2.5
2/0.5	0.5	2.0
3/0.5	0.5	3.0
5/0.5	0.5	5.0

***Table 1:** Optimizing ICAFectin®-mRNA/RNA ratio for adherent cell lines*

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Scaling up/down Transfections

To transfect cells in different cell culture formats, vary the amount of RNA, ICAFectin®-mRNA, cells and medium used, according to **table 2** suggested proportions.

Culture format	Volume of plated cells	Volume of medium during transfection	Opti-MEM volume in RNA dilution tubes (µL)	Added RNA (µg) in H_2O (µL) in RNA dilution tubes	Opti-MEM volume in ICAFectin®-mRNA dilution tubes (µL)	Added volume of ICAFectin®-mRNA to ICAFectin®-mRNA dilution tubes (µL)
96-well	200 µL	100 µL	10	0.05 µg in 1 µL	10	0.3 µL
24-well	1 mL	500 µL	50	0.25 µg in 5µL	50	1.5 µL
12-well	2 mL	1 mL	100	0.5 µg in 10 µL	100	3 µL
6-well	5 mL	2.5 mL	250	1.25 µg in 25 µL	250	7.5 µL
35 mm	5 mL	2.5 mL	250	1.25 µg in 25 µL	250	7.5 µL
60mm	6 mL	3 mL	300	1.5 µg in 30 µL	300	9 µL
100 mm	10 mL	5 mL	500	2.5 µg in 50 µL	500	15 µL
T-25	5 mL	2.5 mL	250	1.25 µg in 25 µL	250	7.5 µL
T-75	10 mL	5 mL	500	2.5 µg in 50 µL	500	15 µL

Table 2: *Optimizing ICAFectin®-mRNA/RNA ratio for adherent cell lines*

Transfection of suspension cells

Forward Transfection Procedure

Use the following procedure to transfect suspension cells in a 24-well format. For other formats, see Scaling up/down Transfections.

Cell preparation

Three hours before transfection, seed 2 x 10^5 cells per well in 200 µL of culture medium with or without serum (depending on the cells).

ICAFectin®-mRNA/RNA complex preparation

All amounts and volume are given to transfect one well in a 24-well format.

1. Dilute 0.5 µg of RNA (in a maximal volume of 5 µL H2O) in 50 µL of Opti-MEM I without serum. Mix gently.

*Note : **Do not use** the serum-free culture medium in which cells were grown*

2. Vortex ICAFectin®-mRNA before use. Dilute 3 µL of ICAFectin®-mRNA in 50 µL of Opti-MEM without serum. Mix gently.

*Note : **Do not use** the serum-free culture medium in which cells were grown.*

3. Combine the diluted ICAFectin®-mRNA (53 µL) with the diluted RNA (55 µL) by pipetting up and down five times and briefly vortexing.
4. Mix gently and incubate 15 minutes at room temperature.
5. Add the entire volume of complexes (108 µL) drop-wise to each well containing cells in 200 µL of medium with or without serum (depending on the cells). Swirl the plate to ensure the homogeneous distribution of complexes. Then, centrifuge the plates 5 minutes at 200g at room temperature.
6. Incubate the cells with the transfection complexes under their normal growth conditions for 3 hours.
7. Add 300 µL of culture medium containing serum to the cells whether cells were transfected in the presence or in the absence of serum and incubate until analysis..

Optimizing transfection

To obtain the highest transfection efficiency, transfect with medium that does not contain serum and after 3 hours add 300 µL of culture medium containing serum to the cells. You can also optimize transfection by varying RNA quantity, ICAFectin®-mRNA amount and cell density.

For initial optimization in 24-well format, use these six ICAFectin®-mRNA/RNA ratios (µL/µg).

Prepare complexes for a single well of a 24-well format as described in **table 3**.

Ratio (µL/µg)	RNA amount (µg) in 5µL H_2O	ICAFectin®-mRNA volume (µL)
2/0.5	0.5	2.0
3/0.5	0.5	3.0
5/0.5	0.5	5.0
4/1	1	4.0
6/1	1	6.0
10/1	1	10.0

Table 3: *Optimizing ICAFectin®-mRNA/RNA ratio for suspension cells.*

Scaling up/down Transfections

To transfect cells in different cell culture formats, vary the amount of RNA, ICAFectin®-mRNA, cells and medium used according to **table 4** suggested proportions.

Culture format	Volume of plated cells	Number of plated cells	Opti-MEM volume in RNA dilution tubes (µL)	Added RNA (µg) in H_2O (µL) in RNA dilution tubes	Opti-MEM volume in ICAFectin®-mRNA dilution tubes	Added volume of ICAFectin®-mRNA to ICAFectin®-mRNA dilution tubes	Volume of medium added after 3 hours
96-well	40 µL	4×10^4	10 µL	0.1 µg in 1 µL	10 µL	0.4 µL	60 µL
24-well	200 µL	2×10^5	50 µL	0.5 µg in 5µL	50 µL	3 µL	300 µL
12-well	400 µL	4×10^5	100 µL	1 µg in 10 µL	100 µL	6 µL	600 µL
6-well	1 mL	10×10^5	250 µL	2.5 µg in 25 µL	250 µL	15 µL	1.5 mL
35 mm	1 mL	10×10^5	250 µL	2.5 µg in 25 µL	250 µL	15 µL	1.5 mL
60mm	1.2 mL	12×10^5	300 µL	3 µg in 30 µL	300 µL	18 µL	1.8 mL
100 mm	2 mL	20×10^5	500 µL	5 µg in 50 µL	500 µL	30 µL	3 mL
T-25	1 mL	10×10^5	250 µL	2.5 µg in 25 µL	250 µL	15 µL	1.5 mL
T-75	2 mL	20×10^5	500 µL	5 µg in 50 µL	500 µL	30 µL	3 mL

Table 4: *Optimizing ICAFectin®-mRNA/RNA ratio for suspension cell lines*